

1 **Bats as potential suppressors of multiple agricultural pests: a case study from**
2 **Madagascar**

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24 **Abstract**

25 The conversion of natural habitats to agriculture is one of the main drivers of biotic change.
26 Madagascar is no exception and land-use change, mostly driven by slash-and-burn
27 agriculture, is impacting the island's exceptional biodiversity. Although most species are
28 negatively affected by agricultural expansion, some, such as synanthropic bats, are capable of
29 exploring newly available resources and benefit from man-made agricultural ecosystems. As
30 bats are known predators of agricultural pests it seems possible that Malagasy bats may be
31 preferentially foraging within agricultural areas and therefore provide important pest
32 suppression services. To investigate the potential role of bats as pest suppressors, we
33 conducted acoustic surveys of insectivorous bats in and around Ranomafana National Park,
34 Madagascar, during November and December 2015. We surveyed five landcover types:
35 irrigated rice, hillside rice, secondary vegetation, forest fragment and continuous forest. 9,569
36 bat passes from a regional assemblage of 19 species were recorded. In parallel, we collected
37 faeces from the six most common bat species to detect insect pest species in their diet using
38 DNA metabarcoding. Total bat activity was higher over rice fields when compared to forest
39 and bats belonging to the open space and edge space sonotypes were the most benefited by
40 the conversion of forest to hillside and irrigated rice. Two economically important rice pests
41 were detected in the faecal samples collected - the paddy swarming armyworm *Spodoptera*
42 *mauritica* was detected in *Mops leucogaster* samples while the grass webworm
43 *Herpetogramma licarsisalis* was detected from *Mormopterus jugularis* and *Miniopterus*
44 *majori* samples. Other crops pests detected included the sugarcane cicada *Yanga guttulata*,
45 the macadamia nut-borer *Thaumatotibia batrachopa* and the sober tabby *Ericeia inangulata*
46 (a pest of citrus fruits). Samples from all bat species also contained reads from important
47 insect disease vectors. In light of our results we argue that Malagasy insectivorous bats have
48 the potential to suppress agricultural pests. It is important to retain and maximise Malagasy

49 bat populations as they may contribute to higher agricultural yields and promote sustainable
50 livelihoods.

51 **1. Introduction**

52 The pervasive conversion of forests for food production is a conspicuous symbol of the
53 Anthropocene (Malhi, 2017). Large swaths of forest have already been cleared for agriculture
54 and the encroachment of natural ecosystems is due to continue as human populations and
55 food demand continue to rise (Giam, 2017). Madagascar holds a unique ensemble of
56 ecosystems and wildlife that is almost unmatched in its biological uniqueness (Goodman and
57 Benstead, 2005). However, despite its high level of endemism and species diversity,
58 Madagascar's forests continue to face one of the highest rates of conversion in the world with
59 approximately 1% of the island's forest cover being cleared each year (Rasolofoson *et al.*,
60 2015; Eklund *et al.*, 2016; Vieilledent *et al.*, 2018). While most Malagasy biodiversity is
61 adversely affected by agriculture-driven habitat modification, some 'winner' species benefit
62 from habitat modification and increase their abundance in agricultural areas. Several of these
63 are insectivorous birds (Martin *et al.*, 2012; Rocha *et al.*, 2015) and bats (López-Baucells *et*
64 *al.*, 2017b) that through the suppression of agricultural pests can provide valuable ecosystem
65 services to local populations (Karp and Daily 2014; Maas *et al.* 2015).

66 Rice (*Oryza* spp.) is one of the most important staple food crops worldwide (Muthayya *et al.*,
67 2014). It is the main crop cultivated by Malagasy subsistence farmers (Kari and Korhonen-
68 Kurki, 2013) throughout the island, and as in numerous other high-biodiversity regions across
69 the tropics, much of the ongoing deforestation is due to agricultural expansion for rice
70 production (McConnell *et al.*, 2004; Neudert *et al.*, 2017). Such a high dependency on rice
71 creates problems when yields are affected by climatic events or pest outbreaks (Harvey *et al.*,
72 2014). Insect rice pests are known to cause severe damage to rice crop yields (Oerke, 2006).

73 Rice crop losses are predominantly caused by Lepidopteran stem borers found across several
74 families such as the Noctuidae, Pyralidae, Tortricidae or Geometridae (Nwilene *et al.*, 2013).

75 Modern day farming techniques incorporate Integrated Pest Management (IPM) to control pest
76 populations (Stenberg, 2017). However, many small-holder farmers in sub-Saharan Africa are
77 unable to access IPM techniques due to lack of financial capital or expertise (Parsa *et al.*, 2014).

78 A sustainable and low cost method to aid pest control and reduce crop losses is through
79 biological control (Bommarco *et al.*, 2013; Naranjo *et al.*, 2015). Biological control, as part of
80 a wider application of integrated pest management practices, can involve insectivorous bats,
81 and has already been proven effective for pecan and rice farms in the USA and Catalonia
82 (Brown *et al.*, 2015; Puig-Montserrat *et al.*, 2015). Multiple lines of evidence support that aerial
83 hawking insectivorous bats provide valuable agricultural pest control services in both
84 temperate and tropical regions (Boyles *et al.*, 2011; Karp and Daily, 2014; Wanger *et al.*, 2014;
85 Brown *et al.*, 2015; Russo *et al.*, 2018). For instance, in the Mediterranean the soprano
86 pipistrelle *Pipistrellus pygmaeus* was found to suppress rice borer moth *Chilo suppressalis*
87 populations through opportunistic foraging (Puig-Montserrat *et al.*, 2015). However, to date
88 most research on tropical bat predation services has focussed on coffee and cacao
89 agroecosystems (Maas *et al.*, 2016), with limited research targeting rice (Wanger *et al.*, 2014).

90 One notable exception comes from Thailand where it was estimated that predation of white
91 backed planthoppers *Sogatella furcifera* by wrinkle-lipped bats *Tadarida plicata* prevents rice
92 crop losses valued at >1.2 million USD (or >26,000 rice meals) each year (Wanger *et al.*, 2014).

93 Numerous bat species (particularly of the Molossidae and Vespertilionidae families) are known
94 to coexist synanthropically by exploring newly available resources. These bat families have
95 been shown to feed on pests (Brown *et al.*, 2015) and to select crops as preferred foraging areas
96 especially during insect pest outbreaks (Lehmkuhl Noer *et al.*, 2012; Taylor *et al.*, 2013a;
97 Davidai *et al.*, 2015). In fact, bats tend to select foraging areas based upon the resources

98 available (Ancillotto *et al.*, 2017), which makes them excellent pest suppressors during
99 seasonal insect pest outbreaks.

100 Large colonies of molossid bats roost in buildings across Madagascar (Razafindrakoto *et al.*,
101 2010; López-Baucells *et al.*, 2017b). However, any potential predation services provided by
102 these colonies are yet to be explored. Forty-two species of insectivorous bats occur in
103 Madagascar, with several species occurring more frequently in anthropogenic landscapes as
104 opposed to forest habitats (Randrianandrianina *et al.*, 2006; Rakotoarivelo *et al.*, 2007). In
105 general, most studies have focused on the dry western region (Goodman *et al.*, 2005; Kofoky
106 *et al.*, 2006; Bambini *et al.*, 2010; Racey *et al.*, 2010; Fernández-Llamazares *et al.*, 2018) as
107 opposed to the humid eastern zone (Randrianandrianina *et al.*, 2006) and only a few studies
108 have tackled habitat selection while none have addressed the potential pest suppressor role in
109 agricultural areas.

110 The DNA metabarcoding of bat faecal pellets can offer valuable insights into the dietary
111 preferences of bats and their potential role as pest suppressors (Bohmann *et al.*, 2014; Swift *et*
112 *al.*, 2018). Recent diet analyses of multiple bat species have detected a wide range of arthropods
113 in bat populations (Galan *et al.*, 2017) including several economically important pest species
114 (Taylor *et al.*, 2017).

115 Here, we combine bioacoustics and DNA metabarcoding to investigate if Malagasy
116 insectivorous bats are foraging within the island's agricultural matrix and if they are consuming
117 important pest species. Specifically, we address the following questions:

- 118 i) How does total bat activity, species (or species-group) activity and assemblage
119 composition change across a rice-dominated agroecosystem landscape? We
120 hypothesise that due to higher insect availability some bats will be more active over

121 rice fields compared to forested sites. We also predict a clear shift in assemblage
122 composition from open to closed landcover types.

123 ii) Which species (or species-groups) are more common within the agricultural matrix?

124 We predict that synanthropic molossids will be particularly abundant in rice fields
125 but we still anticipate some forest associated species to forage outside the forest
126 border.

127 iii) Are bats roosting within the agricultural matrix predated on agricultural insect
128 pests? We expect bats to predate mainly on moths and beetles and we predict that
129 several of these will be agricultural pests of rice and other crops.

130 **2. Methods**

131 **2.1 Study area**

132 Fieldwork was conducted primarily in the peripheral zone surrounding the Ranomafana
133 National Park (RNP) (21°16'S, 47°20'E). The peripheral zone comprises over 160 villages with
134 a population in excess of 50,000 in an area of approximately 500 km² (Kari and Korhonen-
135 Kurki, 2013). Agricultural communities in the region, like many throughout Madagascar,
136 cultivate rice through slash-and-burn agriculture (*tavy*) and irrigated paddies (Peters, 1998;
137 Brooks *et al.*, 2009). The RNP is located between the central highlands and the eastern
138 lowlands and is of particular ecological and economic interest due to its high biodiversity and
139 watershed protection role.

140 **2.2 Bat surveys**

141 Bats were surveyed from November to December 2015 in 54 sites in and around RNP (Fig. 1).
142 Sites were clustered around seven villages (Kelilalina, Tsaratanana, Mangevo,
143 Andriamamovoka, Amboasary, Mandriandry and Tolongoina) and were classified into five
144 landcover categories: irrigated rice fields ($n = 12$), hillside rice fields ($n = 8$), secondary

145 vegetation i.e. fallow agricultural land of mixed successional vegetation ($n = 11$), forest
146 fragment ($n = 9$) and continuous forest in RNP ($n = 15$) (for landcover images and description
147 see supplementary materials Fig. A.1.). Bat activity was recorded using SongMeter SM2BAT+
148 and SM3 autonomous bat detectors (Wildlife Acoustics, Concord, MA, USA). Detectors were
149 secured to a tree at approximately 1.5 m with external SMX-II omni-directional microphones
150 (Wildlife Acoustics, Concord, MA, USA). Detectors were set to record calls continuously from
151 18:00 until 06:00 for three consecutive nights at each locality. Bat activity was sampled for
152 1,956 hours across a total of 147 detector-nights of sampling effort. Detectors were set with a
153 384 kHz sample rate, 12 kHz digital high pass filter, 18 dB trigger level, microphone bias off,
154 and 36 dB gain. We used a 1.0 s trigger window minimum to capture calls prior to the initial
155 trigger.

156 #Figure 1 approximately here#

157 **2.3 Bioacoustic analysis**

158 Recordings were manually classified using Kaleidoscope software version 3.1.7 (Wildlife
159 Acoustics, Concord, MA, USA). We defined a bat pass as a recording of five seconds
160 maximum with at least two pulses with more than 20 dB of difference between the background
161 noise and bat call (Appel *et al.*, 2017) Call sequences were manually identified to species level
162 or left as mixed species groups, or sonotypes, where it was not possible to clearly assign a call
163 to a particular species (Torrent *et al.*, 2018). Call sequences were also identified as feeding
164 buzzes (specific echolocation sequence that a bat uses as it pursues and subsequently catches
165 its prey). We used the frequency of maximum energy or peak frequency (kHz), the start and
166 ending frequencies (kHz), the duration (ms) and the call shape to identify or group species from
167 the existing literature and our own release calls (Fenton *et al.*, 1980; Russ and Bennett, 2001;
168 Kofoky *et al.*, 2009; Goodman *et al.*, 2011; Goodman *et al.*, 2015). Our analysis included a
169 total of 11 sonotypes from the families Emballonuridae, Hipposideridae, Molossidae,

170 Miniopteridae, Myzopodidae and Vespertilionidae. Five sonotypes were classified to species
171 level and the remaining six into sonotype groups (Table 1).

172 #Table 1 approximately here#

173 **2.4 Faecal sample collection**

174 Mist-nets were used to capture bats at roosts in five villages in the RNP area (for sampling
175 details see López-Baucells *et al.*, 2017). Three caves were inspected and surveyed with mist-
176 netting outside of the emergence point (for *Miniopterus* spp. and *Myotis goudoti*). Bats were
177 measured, weighed and identified using keys (Russ and Bennett, 2001; Goodman, 2011). Bat
178 capture and handling was conducted following guidelines approved by the American Society
179 of Mammalogists (Sikes *et al.*, 2011). We kept the bats in cloth bags for an hour until defecation
180 occurred. Faecal pellets were labelled and stored in 2 ml tubes in 95% ethanol and stored in a
181 cool dry space. Of the 322 bats caught, we collected faecal samples from 150 bats. Fifty-eight
182 faecal samples from six species (*Chaerephon atsinanana*, *Mops leucostigma*, *Mormopterus*
183 *jugularis*, *Myotis goudoti*, *Miniopterus manavi*, *Miniopterus majori*) were used for the diet
184 analysis.

185 **2.5 DNA extraction and PCR amplification**

186 The DNA was extracted from the faecal samples using the Norgen Stool Kit following
187 instructions provided by the manufacturers (Norgen Biotek Corp.). Amplification of DNA
188 from the faeces was achieved using the Leray-XT PCR primer set (Wangensteen *et al.*, 2018b),
189 a highly degenerated primer set targeting a 313-bp fragment of the mitochondrial cytochrome
190 c. oxidase subunit I (COI) region. The mlCOIintF-XT primer (5'-
191 GGWACWRGWTGRACWITITAYCCYCC-3') was used as forward primer. This modified
192 version (Wangensteen *et al.*, 2018b) of the mlCOIintF primer (Leray *et al.*, 2013) included two
193 extra degenerate bases (equimolar mixtures of two different bases at a given position) and two

194 inosine nucleotides to enhance its eukaryotic universality. The reverse primer was jgHCO2198
195 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3';(Geller *et al.*, 2013)). The Leray fragment has
196 already been successfully applied to the characterisation of both marine fish gut contents (Leray
197 *et al.*, 2013), marine invertebrates (Siegenthaler *et al.*, 2018) and terrestrial arthropods (Macías-
198 Hernández *et al.*, 2018). A single-PCR step using primers with attached eight-base oligo-tags
199 (Coissac *et al.*, 2012) was used to label different samples in a multiplexed library; moreover a
200 variable number (2, 3 or 4) of fully degenerate positions (Ns) was added at the beginning of
201 each primer, in order to increase variability of the amplicon sequences (Guardiola *et al.*, 2015)
202 .The PCR mix recipe included 10 µl AmpliTaq gold 360 master mix (Applied Biosystems), 3.2
203 µg Bovine Serum Albumin (Thermo-Scientific), 1 µl of each of the 5 µM forward and reverse
204 tagged-primers, 5.84 µl water and 2 µl extracted DNA template (~ 5 ng µl⁻¹). The PCR profile
205 included an initial denaturing step of 95 °C for 10 min, 35 cycles of 94 °C for 1 min, 45 °C for
206 1 min and 72 °C for 1 min and a final extension step of 72 °C for 5 minutes. After a quality
207 check of all amplicons by electrophoresis, the tagged PCR products (including a negative
208 control) were pooled into a multiplexed sample pool and purified using MinElute columns
209 (Qiagen). An Illumina library was subsequently built from these pools, using the NextFlex
210 PCR-free library preparation kit (BIOO Scientific). The library was sequenced on an Illumina
211 MiSeq platform using v3 chemistry (2x250 bp paired-ends), as part of a multiplexed run
212 including ten other unrelated libraries.

213 **2.7 Bioinformatic analyses**

214 Bioinformatic analyses were performed using the OBITools metabarcoding software suite
215 (Boyer *et al.*, 2016). Read quality assessment was performed with FastQC and only paired-end
216 reads with phred quality score > 40 was retained. Demultiplexing and primer removal were
217 achieved using ngsfilter. Obigrep was applied to select all aligned reads with a length between
218 303-323 bp and without ambiguous bases. Obiuniq was used to dereplicate the reads and the

219 uchime-denovo algorithm (Edgar *et al.*, 2011) implemented in VSEARCH (Rognes *et al.*,
220 2016) was used to remove chimeric sequences. Amplicon clustering was performed using the
221 SWARM 2.0 algorithm (Mahé *et al.*, 2015) with a distance value of $d=13$, which offers a
222 conservative solution to the high variability of the COI gene (Siegenthaler *et al.*, 2018).
223 Taxonomic assignment of the representative sequences for each molecular operational
224 taxonomic unit (MOTU) was performed using the ecotag algorithm (Boyer *et al.*, 2016), using
225 a local reference database (Wangenstein *et al.*, 2018b) containing filtered COI sequences
226 retrieved from the BOLD database (Ratnasingham and Hebert, 2007) and the EMBL repository
227 (Kulikova *et al.*, 2004). This algorithm uses a phylogenetic approach to assign sequences to
228 the most reliable monophyletic unit, so that sequences are assigned to different taxonomic
229 ranks, depending on the density of the reference database. The data was refined by removing
230 contaminations of marine origin (originated by tag-switching from other multiplexed libraries
231 in the sequencing run). A minimum abundance filter of 5 total reads was used to avoid false
232 positives and low frequency noise (De Barba *et al.*, 2014; Wangenstein and Turon, 2017). This
233 pipeline, with little variations, has been previously used for analysing metabarcoding data for
234 the same COI marker in a variety of systems (e.g. Wangenstein and Turon, 2017; Macías-
235 Hernández *et al.*, 2018; Siegenthaler *et al.*, 2018; Wangenstein *et al.*, 2018a; Wangenstein *et al.*,
236 2018b). The resulting data has been deposited on Mendeley Data ([dataset] Kemp *et al.*,
237 2018)

238 **2.8 Statistical analysis**

239 Bat activity was defined as the total number of bat passes per night from all species as well as
240 for each sonotype (Torrent *et al.*, 2018). As appropriate for count data, negative binomial or
241 Poisson generalized linear mixed models (GLMMs) with a log link function were used to
242 model the relationship between bat activity and landcover type (continuous forest, forest
243 fragments, secondary vegetation, hillside rice and irrigated rice) (Burnham and Anderson,

244 2003). Species with less than 300 recordings were not used in the analysis due to a lack of
245 model convergence. Since preliminary analyses suggested that the count data were
246 overdispersed, we accounted for this overdispersion by using a Poisson or negative binomial
247 regression in *glmer* or *glmmADMB* and adding a random intercept of “Site” nested within
248 “Location” (Bates, 2010).

249 Numbers of bat passes were positively correlated with feeding buzzes (Table A.1). We
250 therefore only used the larger bat passes dataset for modelling as a proxy for feeding activity
251 (Torrent *et al.*, 2018). Moran’s *I* test showed that there is no residual spatial autocorrelation
252 between sites (Table A.2). The difference in assemblage structure between landcover types was
253 analysed using the analysis of dissimilarity test *adonis*. It was visualised through a non-metric
254 multidimensional scaling (NMDS), based on a Bray-Curtis dissimilarity matrix, using sonotype
255 activity data per site. We analysed and presented the data using R statistical software 3.4.1 (R
256 Development Core Team, 2017) with the packages: tidyverse (Wickham, 2016), lme4 (Bates
257 *et al.*, 2014), glmmADMB (Skaug *et al.*, 2015) and vegan (Oksanen *et al.*, 2013).

258 The relative abundance of MOTU reads for prey items (excluding predator reads and
259 normalized to 10,000 total prey reads per sample) was calculated for all prey MOTUs. The
260 relative abundances per faecal sample for all prey MOTUs were then averaged per bat species.
261 We then grouped the MOTU sequences by arthropod orders and highlighted the pest and
262 disease transmitting insect species, alongside any species or genera that we suspected to have
263 a potential pest status.

264 **3. Results**

265 **3.1 Bat activity**

266 We recorded a total of 9,569 bat passes, of which 1,643 (17 %) were identified to species level
267 (*Hipposideros commersoni*, *M. manavi*, *M. goudoti*, *Myzopoda aurita*, *Paraemballonura*
268 *atrata*), 2,261 (24 %) were identified to sonotypes of two species (*Miniopterus gleni*/*M.*
269 *majori*, *Scotophilus robustus*/*M. gleni*, *S. robustus*/*M. jugularis*, *Otomops*
270 *madagascariensis*/*Tadarida fulminans*), and 5,665 (60 %) were attributed to sonotypes
271 Molossidae 1 (Mo1: *C. atsinanana*, *M. leucostigma*, *M. jugularis*, *Taphozous mauritanus*) and
272 Vespertilionidae/Miniopteridae 1 (VMi1: *M. gleni*, *M. majori*, *M. manavi*, *Miniopterus*
273 *soroculus*, *Neoromicia matroka*, *Pipistrellus hesperidus*, *Pipistrellus raceyi*). In total 1,013
274 feeding buzzes were recorded, with Mo1 accounting for 389 (38 %) of feeding buzzes, VMi1
275 for 334 (33 %) and *P. atrata* for 102 (10 %).

276 Bat activity was highest in hillside rice with a mean of 197 passes/night and more than double
277 that of the next landcover type with more bat activity - irrigated rice at 89 passes/night (Table
278 2). Overall bat activity in both types of rice field, hillside and irrigated, was higher than activity
279 levels in continuous forest (Table A.3). According to pairwise comparisons (Table A.4), total
280 bat activity over hillside rice was higher than in continuous forest ($p < 0.01$) and forest fragments
281 ($p < 0.05$) whereas activity in irrigated rice was only higher than continuous forest ($p < 0.01$).

282 In hillside and irrigated rice, Mo1, VMi1, *M. goudoti* and *M. gleni*/*M. majori*, had significantly
283 higher activity compared to continuous forest while *O. madagascariensis*/*T. fulminans* was
284 higher in hillside rice compared to continuous forest. In continuous forest and forest fragments,
285 *P. atrata* and *M. goudoti* had the highest mean bat passes/night, respectively (Fig. 2).

286 #Table 2 approximately here#

287 #Figure 2 approximately here#

288 **3.2 Assemblage composition**

289 Assemblage composition varied between landcover type (adonis: $r^2 = 0.253$; $p = 0.001$). This
290 was corroborated by the NMDS ordination which revealed distinct patterns of dissimilarities
291 in assemblage composition between the five landcover classes (Fig. 3). The NMDS had a final
292 stress value of 0.12 conveying a good representation of the data along the represented
293 dimensions.

294 #Figure 3 approximately here#

295 **3.3 Presence of insect pests in faecal samples (DNA metabarcoding)**

296 We obtained a total number of 655,205 MOTU reads from all samples. 43.5% (285,978) of the
297 reads were attributed to bats while 5.3% (34,599) of the reads were assigned to arthropods.
298 Overall, when looking at the insect orders found in the faecal samples, the highest average
299 relative abundance of MOTU reads found were of Coleoptera, Lepidoptera, Ephemeroptera,
300 Diptera and Hemiptera (Table 3). All the bats species sampled fed on, at least, 11-13 orders of
301 arthropods.

302 #Table 3 approximately here#

303 In 58 bat faecal samples we found six known pest species, seven insect vectors of human
304 diseases and 17 potential pest taxa (Table A.5). Of the known agricultural pests found in the
305 faecal samples, two economically important rice pest species were found – the paddy swarming
306 caterpillar *Spodoptera mauritia* in *M. leucogaster* and the grass webworm *Herpetogramma*
307 *licarsisalis* in *M. jugularis* and *M. majori*. Other crops pests detected were: the black twig borer
308 *Xyleborus ferrugineus* a pest of coffee; the sugarcane cicada *Yanga guttulata*; the macadamia
309 nut-borer *Thaumatotibia batrachopa* and the sober tabby *Ericeia inangulata* a pest of citrus
310 fruits. Potential pest species and genera, from the order Lepidoptera, were found in all bat
311 species. In particular: *Mythimna* sp. – a genus containing the rice armyworm *Mythimna*
312 *unipuncta*; *Emmalocera* sp. – a genus containing a sugarcane root borer *Emmalocera*

313 *depressella*; and *Cydia choleropa* – a sister species of the codling moth *Cydia pomonella* a pest
314 of apples and pears.

315 **Discussion**

316 Large colonies of, predominantly, molossid, vespertilionid and miniopterid bats, were found
317 to be preferentially selecting the rice fields surrounding the RNP. Six species of bats were
318 shown to have fed upon economically important insect pests such as the paddy swarming
319 caterpillar (*Spodoptera mauritia*) and the Grass webworm (*Herpetogramma licarsisalis*). In
320 agreement with Puig-montserrat *et al.* (2011) and Wanger *et al.* (2014) insectivorous bats,
321 particularly molossids, are likely to be preferentially selecting rice fields for foraging and
322 feeding upon rice crops pests and other economically important insects.

323 *Bat activity across landcover types.*

324 The highest overall mean activity was found in hillside rice followed by irrigated rice and
325 secondary vegetation (Table 2). Hillside rice has markedly lower yields compared to lowland
326 irrigated rice. Water and nutrient run-off impact the growth of upland rice. A lack of water and
327 nutrient retention in the rice crop makes it more susceptible to insect pest infestations. This
328 may be one reason why we recorded the highest activity in hillside rice. However, it is also
329 possible that there was an altitudinal detection bias as hillside rice and secondary vegetation
330 sites were on open hillsides with little vegetation and facing large valleys (Collins and Jones,
331 2009). Both sites, however, were found at similar altitudes and had markedly different results
332 (Table 2, Table A.3). Irrigated rice sites, on the other hand, are found at the bottom of valleys.
333 Despite the possible altitudinal bias, activity within irrigated rice was the second highest of the
334 landcover types (Table 2, Table A.3). Intensive rice agriculture harbours high densities of
335 insect pests which provide an excellent resource for insectivorous bats. Insects form swarms,
336 especially tympanic moths (Noctuidae, Crambidae and Pyralidae), during mating and

337 emergence, which bats are able to opportunistically prey upon (McCracken *et al.*, 2012;
338 Malmqvist *et al.*, 2018).

339 The open space group, Mo1, was the most active overall and over hillside rice (Figure 2, Table
340 2). This suggests that open-space aerial hawkers are important agents of pest suppression in
341 the rice-dominated landscape surrounding the RNP and potentially throughout much of
342 Madagascar's agroecosystems. Further research and action is required to improve the
343 knowledge of bats dietary preferences, both temporally and spatially. The reputation of these
344 bats among local communities needs to be improved, especially as they form large colonies in
345 public buildings (López-Baucells *et al.*, 2017b).

346 The results conform to the notion that molossids (in addition to *Taphozous mauritanus*), which
347 are open-space aerial hawkers, commute and forage at higher altitudes than other families (Lee
348 and McCracken, 2002; McCracken *et al.*, 2008; Taylor *et al.*, 2013b). Open space foragers
349 have a high wing loading ratio (fast flight; low manoeuvrability) which suggests that they do
350 not use cluttered sites and this explains their low detection in forested areas (Schnitzler and
351 Kalko, 2001).

352 The sonotype VMi1, comprised of three vespertilionids and three miniopterids, was found to
353 be the most active in irrigated rice fields and the second most active overall (Figure 2, Table
354 2). The species in this sonotype forage as edge-space aerial-hawkers (Verboom and Huitema,
355 1997; Taylor *et al.*, 2013b). The mosaic of vegetation and fruit trees, rivers and streams, paths,
356 terraces and anthropomorphic structures within the vicinity of the rice fields may provide this
357 group with the required heterogeneity or "edge" habitat to forage (Monck-Whipp *et al.*, 2018).
358 This is important for the contextualization of our results as edge-space foragers are known to
359 predate upon insect pests within agroecosystems (Taylor *et al.*, 2013a; Taylor *et al.*, 2013b;
360 Brown *et al.*, 2015; Puig-Montserrat *et al.*, 2015)

361 The edge-clutter species, *Myotis goudoti* and *Paraemballonura atrata*, were previously
362 captured in forest in the same region as our study (Goodman *et al.*, 2014). We recorded both
363 species at relatively high levels in irrigated rice, forest fragments and continuous forest sites.
364 Although post hoc tests showed no significant differences, this activity shows that these species
365 are selecting lowland irrigated rice and forest for foraging. The NMDS plots show that both
366 species are strongly associated with forest sites (Fig. 3). NMDS axis 2 shows *P. atrata* is more
367 associated with rice fields than *M. goudoti*. The fact that these edge-clutter species, *P. atrata*
368 and *M. goudoti*, switch between open and closed sites highlights the importance of retaining
369 forest nearby for roost provision and maintaining bat populations. The paucity of available
370 roosts for bats in rice dominated landscapes is certainly an issue and one that requires
371 addressing as a sustainable solution to crop losses. Installing bat houses and improving
372 landscape heterogeneity are ways to address the lack of suitable roosts available (Flaquer *et*
373 *al.*, 2006; Lindell *et al.*, 2018; Monck-Whipp *et al.*, 2018).

374 Additionally, we recorded two charismatic, endemic and difficult to catch species - *Myzopoda*
375 *aurita* and *Hipposideros commersoni*. The eastern sucker-footed bat *Myzopoda aurita* was
376 recorded in hillside and irrigated rice and in secondary vegetation (Table 2). This species roosts
377 in the furled-up leaves of the traveller's palm *Ravenala madagascariensis* which can grow in
378 open areas of vegetation or forest. Commersoni's horseshoe bat *Hipposideros commersoni* is
379 the largest insectivorous bat in Madagascar, listed as Near Threatened (Andriafidison, 2008),
380 and it was mainly recorded in hillside rice (Table 2). The echolocation of *Hipposideros*
381 *commersoni* (high duty cycle echolocation) is extremely efficient for hunting in cluttered
382 spaces. The bat may be roosting in the remnant forests and foraging in the adjacent hillside
383 rice. The rarity of both species might limit their predation services but their high association
384 with forest habitat qualifies them as good indicator taxa for the evaluation of habitat
385 disturbance.

386 As expected, from the NMDS plots, the assemblage composition in the landscape shows that
387 there was a turnover of species and sonotypes (Fig. 3). One can see a gradient from irrigated
388 rice to continuous forest, from left to right. The open space foragers (Mo1 and *O.*
389 *madagascariensis*/*T. fulminans*) occupy the left side of NMDS axis 1 while the clutter and
390 edge-space foragers (*M. goudoti* and *P. atrata*) occupy the right side of the plot, illustrating the
391 foraging preferences of the aforementioned guilds (Schnitzler and Kalko, 2001). Hillside rice
392 and secondary vegetation almost entirely overlap which illustrates the similarity of these sites
393 in terms of species assemblage.

394 *Diet analysis and implications of bat foraging behaviour*

395 The DNA metabarcoding results illustrate that insectivorous bats feed on a wide range of prey
396 including a number of economically important insect pest species that affect a range of crops
397 in addition to insect disease vectors (see supplementary materials Table A.5.). The results of
398 this study, therefore, show the potential role of insectivorous bats in suppressing economically
399 important insects in agricultural landscapes.

400 We found that the sonotypes that were preferentially selecting rice fields were also the most
401 important contributors to pest suppression in rice fields. For example, *M. leucogaster* and *M.*
402 *jugularis* from the Mo1 sonotype were found to have fed upon the rice pests *Spodoptera*
403 *mauritica* and *Herpetogramma licarsisalis*, respectively (Table A.5). Greater pest suppression
404 leads to greater yields and less reliance upon slash and burn agriculture, or *tavy* (Styger *et al.*,
405 2007). This form of agriculture is environmentally damaging and encroaches upon forests when
406 fallow lands are no longer fertile. Forest fragments still offer valuable refuges for certain
407 species, yet insectivorous bats generally prefer rice fields for foraging. By identifying the most
408 active sonotypes and how they change across different land-uses we can begin to understand
409 the level of pest suppression that bats provide to agricultural landscapes.

410 It is important to note that the fieldwork only spanned a short amount of time (approximately
411 three days per locality). The research therefore does not reflect the seasonal and spatial
412 variation of bat diets nor do the results intentionally follow peaks in insect populations.
413 Additionally, although we have identified bat predation on predatory arthropods that can
414 potentially contribute to the suppression of agricultural pests (e.g. spiders - order Araneae -
415 were identified in the diet of *M. goudoti*; Table 3) we did not explore the effects of intra-guild
416 predation on herbivorous arthropods. Since most Malagasy bats are predominantly aerial
417 feeders we anticipated that bat predation on non-flying arthropods would be limited. We
418 suggest that future research should try to investigate the effects of intra-guild predation and
419 any potential cascading effects on the abundance of agricultural pests and on rice yield.
420 Furthermore, despite the fact that our study focussed on a rice-dominated agroecosystem, it is
421 important to note that the pests of other crops found in bat faeces illustrates the global potential
422 of bats as pest suppressors. Further research quantifying the role of bats as pest suppressors in
423 Madagascar is urgently needed as they: receive little protection from Malagasy legislation; fall
424 under game species regulations i.e. they are not actively protected; many are data deficient;
425 and there is little appreciation of their role in ecosystem services (Racey *et al.*, 2010).

426 **Conclusions**

427 Deforestation and habitat loss due to agricultural expansion are the primary driver of
428 biodiversity loss in Madagascar. The need for agricultural expansion to compensate for crop
429 losses is exacerbated by climatic extremes and insect pest outbreaks. We found that Malagasy
430 insectivorous bats have the potential to suppress these outbreaks as they predate upon insect
431 pests. Therefore, retaining and maximising bat populations across the island's agricultural
432 landscapes can contribute to higher agricultural yields and help promote sustainable
433 livelihoods. Provision of artificial roosts such as bat-boxes (Puig-Montserrat *et al.*, 2015;
434 López-Baucells *et al.*, 2017a) and increased landscape heterogeneity is an important

435 consideration for agricultural and conservation planning, specifically for open and edge-
436 foragers. Since some cave-dwelling bat species (i.e. *Miniopterus manavi*, *Miniopterus majori*,
437 and *Myotis goudoti*) were also predated on insect pests, appropriate conservation legislation
438 and cave protection initiatives (i.e. regulation of the harvesting of guano and cave tourism) are
439 essential to keep their populations stable. Further research and action is required to improve
440 the knowledge of bat dietary preferences, following pest outbreaks both temporally and
441 spatially, while improving the reputation of bats among local communities.

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453 **Figure and table captions**

454 Figure 1. Map of sampling sites within and surrounding the Ranomafana National Park,
455 Madagascar.

456 Figure 2. Mean bat activity per night per sonotype (>300 passes) for each landcover type,
457 with standard errors. See Table 1 for sonotype abbreviations.

458 Figure 3. NMDS plot showing community assemblage of sonotypes (in text) relative to
459 sampling sites (coloured dots – corresponding to landcover type). See Table 1 for sonotype
460 abbreviations.

461 Table 1. List of species known to occur in the region incorporating Ranomafana National
462 Park with sonotypes created from mean peak frequency ranges from the existing literature.

463 Table 2. Mean bat passes (\pm SD) per night per sonotype across each landcover type.
464 Significant differences to continuous forest from generalised linear mixed models highlighted
465 in bold.

466 Table 3. Average relative abundance of MOTU reads per 10,000 reads for six bat species
467 (number of samples in brackets) grouped by arthropod order. See Table A.5 for insect pest
468 and disease vector species and genera.

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